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14. ABSTRACT

Radiotherapy is a prevalent modality for the treatment of prostate tumor. Although radiation is capable of eradicating localized prostate tumors, nearly 30% of patients treated with potentially curative doses relapse at the sites of irradiation. Therefore, there is an imperative need to improve the success rate of radiotherapy for PCa. This proposal is focused on a role of 12-lipoxygenase (LOX) in modulating the radiation response of PCa cells. 12-LOX catalyzes the formation of 12(S)-hydroxyeicosatetraenoic acid (HETE). Our studies suggest an involvement of 12-LOX in radioresistance of PCa cells. It is our hypothesis that an increase in 12-LOX expression/activity may lead to an increased resistance in tumors to radiation treatment. We also hypothesize that VEGF is an important intermediary for 12-LOX mediated radioresistance in PCa. We intend to define the role of 12-LOX in radioresponse in PCa. 12-LOX will be overexpressed in LNCaP and DU145 cells. Then we will study whether an increase in 12-LOX expression in LNCaP and DU145 cells can enhance their resistance to radiotherapy. We also propose to study whether VEGF is required by 12-LOX to enhance PCa radioresistance through blockade of VEGF activity with a neutralizing antibody. Finally, we will evaluate whether BHPP, a 12-LOX inhibitor, can be used to sensitize prostate tumors to radiotherapy.

15. SUBJECT TERMS
Prostate cancer, radiotherapy, 12-lipoxygenase, apoptosis, angiogenesis

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INTRODUCTION

Prostate cancer (PCa) is one of most common cancers affecting American men. Radiotherapy is a prevalent modality for the treatment of prostate tumor. Although radiation is capable of eradicating localized prostate tumors, nearly 30% of patients treated with potentially curative doses relapse at the sites of irradiation. Therefore, there is an imperative need to improve the success rate of radiotherapy for PCa.

This proposal is focused on acquisition of resistance to radiotherapy by tumor cells, specifically the potential role of 12-lipoxygenase (LOX) in modulating the radiation response of PCa cells. 12-LOX catalyzes the formation of 12(S)-hydroxyeicosatetraenoic acid (HETE) and it has been implicated in PCa growth and progression. Our studies suggest an involvement of 12-LOX in radioresistance of PCa cells. It is our hypothesis that an increase in 12-LOX expression/activity may lead to an increased resistance in tumors to radiation treatment. Conversely, downregulation of 12-LOX expression or activity can sensitize PCa cells to radiotherapy. We also hypothesize that VEGF is an important intermediary for 12-LOX mediated radioresistance in PCa. Here we propose to expand our study on the role for 12-LOX in radioresponse in PCa. 12-LOX will be overexpressed in LNCaP and DU145 cells. Then we will study whether an increase in 12-LOX expression in LNCaP and DU145 cells can enhance their resistance to radiotherapy. We also propose to study whether VEGF is required by 12-LOX to enhance PCa radioresistance through blockade of VEGF activity with a neutralizing antibody. Finally, we will evaluate whether BHPP, a 12-LOX inhibitor, can be used to sensitize prostate tumors to radiotherapy. The following specific aims are proposed:

Aim 1. Expand the study on the role of 12-LOX in radioresponse in PCa cells.

Aim 2. Determine whether or not stimulation of VEGF is required by 12-LOX to enhance radioresistance in vitro and in vivo.

Aim 3. Evaluate whether or not 12-LOX inhibitor BHPP can sensitize prostate tumors to radiation in vivo.

BODY OF REPORT

KEY RESEARCH ACCOMPLISHMENT

1 provisional patent application filed
3 review articles published
3 research article published
3 abstracts published

PROGRESS

Task 1. Expand the study of the role for 12-LOX in radioresponse in prostate cancer cells. Months 1 - 18:

In this aim, the regulation of 12-LOX levels by IR will be studied in a number of prostate cancer cell lines. The radiosensitizing effects of 12-LOX inhibitors in more PCa cell lines and whether 12(S)-HETE can protect them from radiation will be studied. This task has been largely completed, with findings summarized below.

Treatment of PC-3, LNCaP and DU 145 human prostate cancer cells with different concentration of 12-LOX inhibitors (Baicalein, BHPP) or different doses of radiation resulted in a concentration/dose dependent decrease in the clonogenic survival of the cells. (Figure 1) The radiation and 12-LOX inhibitor sensitivity of the cells differed significantly.

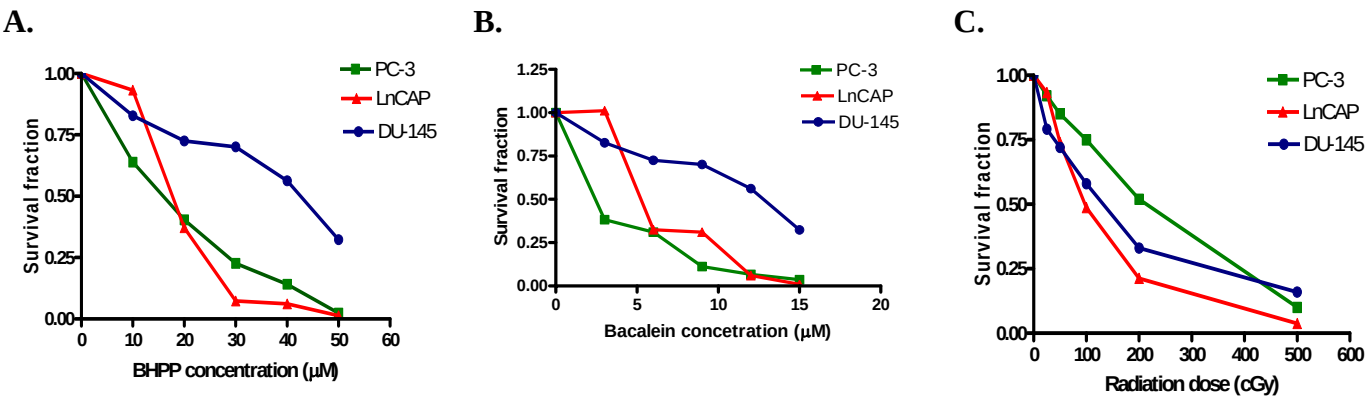

Figure 1. *Clonogenic survival of human prostate cancer cells PC-3, LNCaP and DU 145 treated with BHPP (A), Baicalein (B) and radiation (C).*

To study whether or not radiation regulates 12-LOX, we subjected LNCaP cells to radiation of different doses and cultured in serum containing media (RPMI1640-10%FBS) for 16 h. LNCaP cells were selected because they express 12-LOX consistently in culture. As shown in **figure 2**, low-dose radiation (200 cGy) increased the protein level of 12-LOX, suggesting that the gene expression of 12-LOX was stimulated by low dose radiation. Interestingly, at higher doses (400 and 1600 cGy), the steady state levels of 12-LOX were reduced. The reduction of 12-LOX level is not due to cell death because we did not notice any significant cell death 16 h after irradiation at doses indicated. The drastic changes in 12-LOX levels as a function of radiation imply that 12-LOX is probably involved in radiation response.

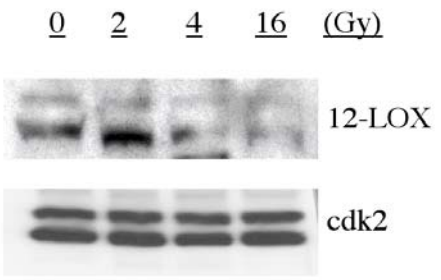

***Figure 2**. Effect of radiation on 12-LOX expression in prostate carcinoma LNCaP cells. Note the stimulation of 12-LOX expression by low dose radiation (200 cGy) but at higher doses, 12-LOX expression was reduced (400 cGy and 1600 cGy). The level of cdk2 is included for reference for sample loading.*

To determine whether 12-LOX plays a role in radioresponse of carcinoma cells, we used a panel of PC-3 cell sublines that were stably transfected with an expression construct of platelet-type 12-LOX. The isolated clones had an increased 12-LOX expression and 12(S)-HETE biosynthesis. Next, we examined the effects of increased expression of 12-LOX on colony formation of carcinoma cells after radiation. As shown in **Figure 3**, nL8, a 12-LOX overexpressing clone, presented strong radioresistance when compared to its vector control, neo-α (**Figure 3A**), as indicated by enhanced clonogenic survival. Regression analysis indicated a significant difference in radioresistance between nL8 and neo-σ ($P < 0.01$) (**Figure 3 B**). The data suggest that increased expression or activity of 12-LOX enhances radioresistance in prostate carcinoma cells.

A **B**

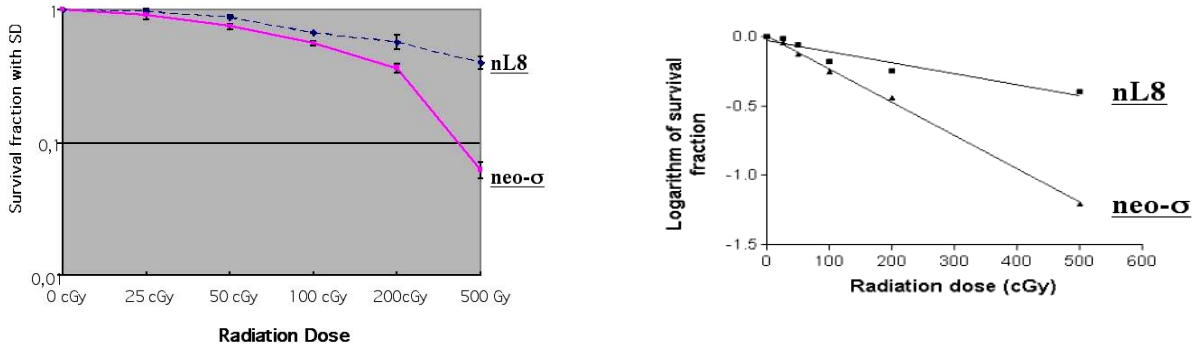

***Figure 3**. 12-Lipoxygenase enhances radioresistance of PC-3 cells as indicated by colony formation assay. A, Increased clonogenic survival by enhanced expression of 12-LOX in PC-3 cells. nL8, a 12-LOX overexpressing clone of PC-3 cells; neo-σ, vector control. B, Regression analysis. P< 0.01.*

Next we examined whether inhibition of 12-LOX can modulate the radioresponse of PCa cells. First we examined the effect of baicalein, a select inhibitor of 12-LOX, on radioresponse of androgen dependent LNCaP cells. We treated LNCaP cells with 7.5 μM baicalein for 2 hrs before initiation of radiation. As shown in **figure 4A**, baicalein and IR, when combined, have super additive or synergistic inhibitory effect on the colony formation of LNCaP cells. Regression analysis indicates that combined treatment of LNCaP cells with radiation and baicalein has significant super-additive or synergistic effect ($P < 0.05$) (**Figure 4B**).

A **B**

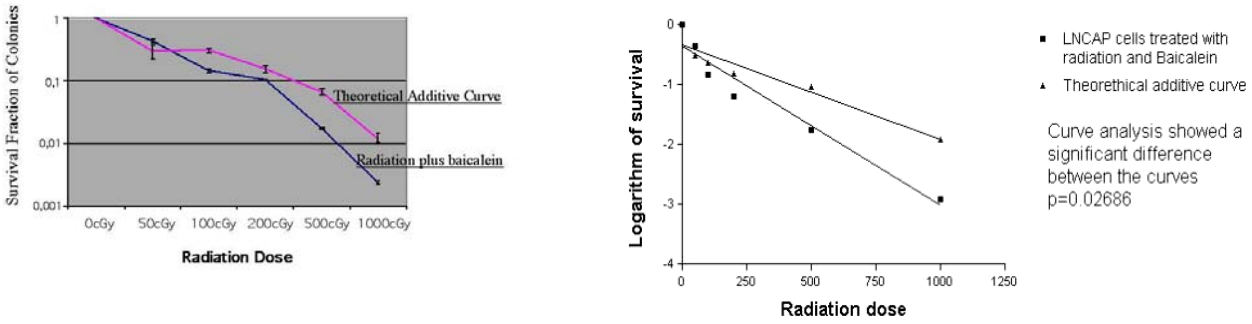

Figure 4*. Radiosensitization of LNCaP cells by a 12-LOX inhibitor, baicalein.* ***A.*** *12-LOX inhibitor baicalein sensitizes LNCaP cells to IR as indicated by colony formation assay. Refer to the General Method in section D for detailed description of the calculation of theoretical additive curve and other statistical calculation.* ***B.*** *Regression analysis. P = 0.02688.*

Next we studied whether baicalein also sensitizes androgen-independent PCa cells to radiation therapy as it did in LNCaP cells. PC-3 cells were treated with 7.5 μM baicalein for two hours before initiation of radiation. As shown in **figure 5 A and B**, baicalein and radiation, when combined, have super additive or synergistic inhibition on the colony formation of PC3 cells ($P < 0.01$). The data suggest that inhibitor of 12-lipoxygenase also sensitizes androgen independent PC-3 cells to radiation.

A **B**

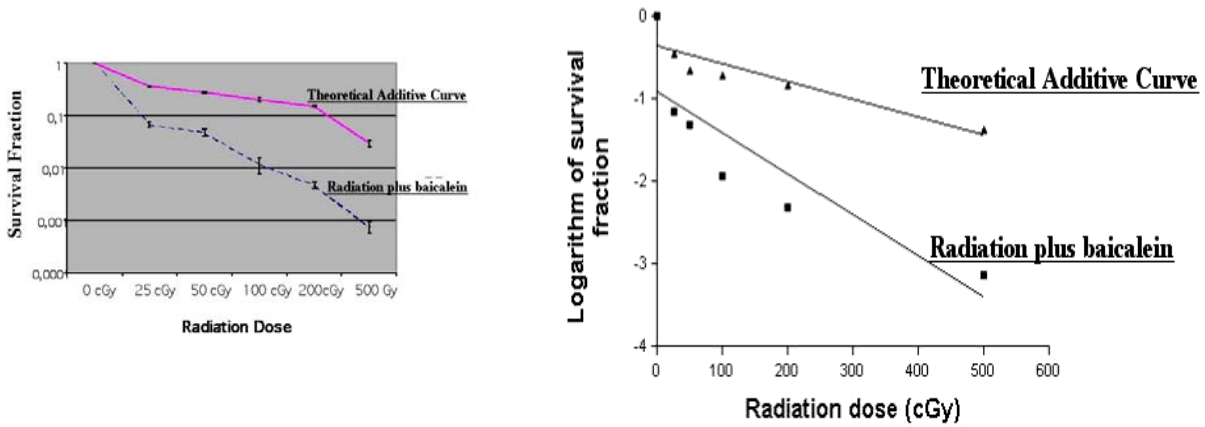

Figure 5*. Radiosensitization of androgen independent PC-3 cells by baicalein.* ***A.*** *12-LOX inhibitor baicalein sensitizes PC3 cells to radiation as indicated by colony formation assay.* ***B.*** *Regression analysis. P = 0.0086.*

The main stable arachidonate product of 12-LOX is 12(S)-HETE. To study whether or not 12(S)-HETE modulates radioresistance of carcinoma cells, we treated PC-3 cells with graded levels of baicalein (0, 3, 6, 9, 12, and 15 μM), in the presence or absence of 300 nM of 12(S)-HETE, for 2 h before irradiation (200 cGy). As shown in **Figure 6 A**, baicalein sensitized PC-3 cells to radiation in a dose dependent manner. The radiosensitization of PC-3 cells by baicalein was completely abolished by exogenously added 12(S)-HETE (**Figure 6 A and B**, $P < 0.01$). Therefore, radiosensitization of PC-3 cells by baicalein is dependent on the *absence* of 12(S)-HETE. The results further suggest the involvement of the 12-LOX activity in radioresistance of prostate carcinoma cells.

A *B*

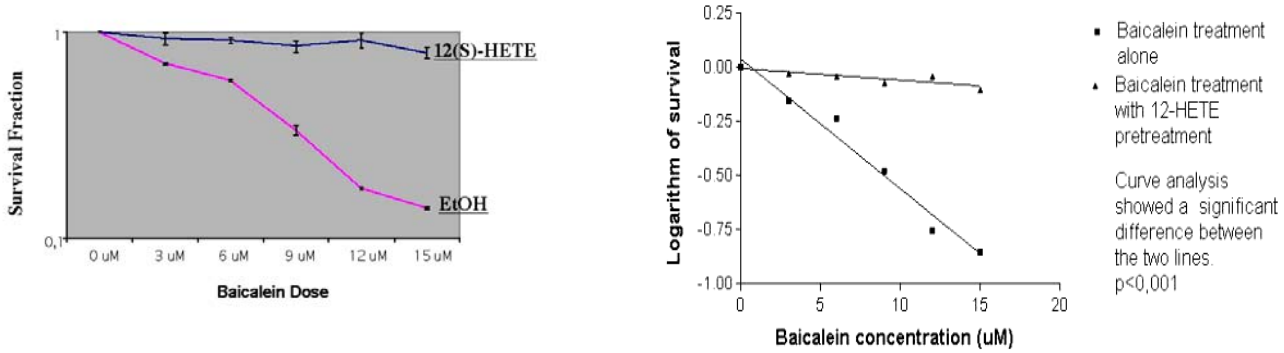

Figure 6*. Radiosensitization of PC-3 cells by baicalein was abolished by exogenously added 12(S)-HETE.* ***A****. Attenuation of baicalein radiosensitization of PC-3 cells by 12(S)-HETE as indicated by colony formation assay. The radiation dose used was 200 cGy.* ***B****. Regression analysis. P < 0.001.*

To study whether 12-LOX inhibitors can also sensitize normal prostate epithelial cells to radiation, we treated human normal prostate epithelial cells (purchased from Clonetics, San Diego, CA) with 7.5 μM baicalein 2 h before radiation (800 cGy). The cells are harvested 36 h after radiation for evaluation of apoptosis using a commercial flow cytometric assay kit based on TUNEL staining (APO-DIRECT, Pharmingen, San Diego, CA). We use apoptosis, rather than clonogenic survival, as the end point for potential radiosensitization of normal prostate epithelial cells by 12-LOX inhibitors. The rationale is that unlike prostate cancer cells, normal prostate cells have limited ability to proliferate and form colonies. As shown in **Figure 7** and **Figure 8**, the presence of baicalein did not potentiate radiation-elicited apoptosis either in normal prostate epithelial cells or in human normal skin fibroblast. The lack of radiosensitization by 12-LOX inhibitor in normal prostate epithelial cells may be due to the low or absence of 12-LOX expression
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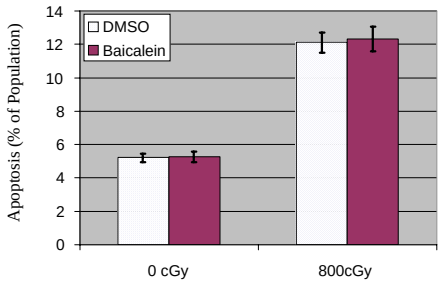


Figure 7*. Lack of radiosensitization of baicalein, a 12-LOX inhibitor, in normal prostate epithelial cells. Note the increase in apoptosis after radiation (800 cGy) and the absence of effect of baicalein treatment on apoptosis, regardless of radiation.*

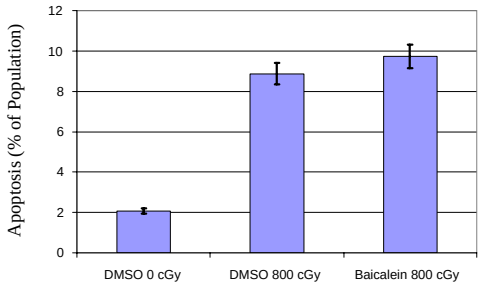


Figure 8*. Lack of radiosensitization of baicalein in normal human skin fibroblast.*

Since 12-LOX inhibitors can induce apoptosis, radiosensitization of tumor cells by baicalein is likely mediated by potentiation of apoptosis. To study this possibility, we evaluated the level of cleaved caspase-3, the activated form of caspase-3. As show in the **figure 9**, combined treatment of A431 cells had highest level of caspase-3 activation.

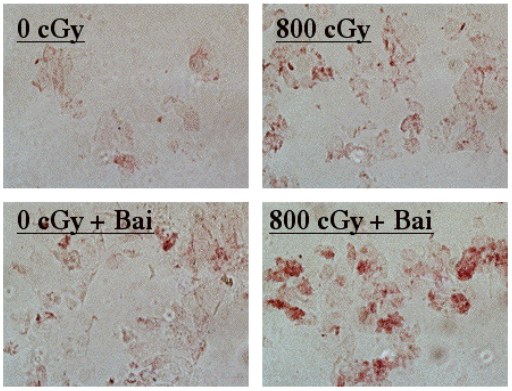


Figure 9. *Levels of cleaved caspase-3 16 h after baicalein and radiation treatment. Cells were fixed and immunostained for cleaved (activated) caspase-3 using standard ABC procedure. Blown staining (dark spots if black and white print) indicates positive staining.*

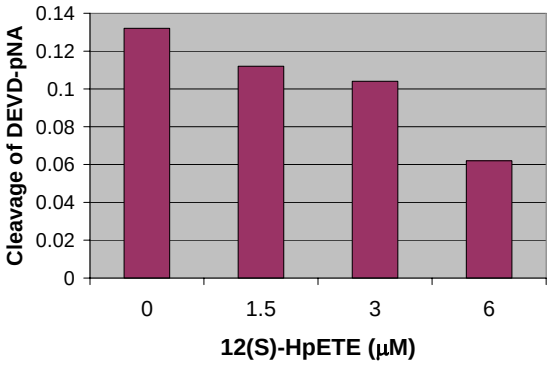


Figure 10*. Inhibition of Caspase-3 activity by 12(S)-HpETE. Active caspase-3 (purchased from Biomol) were incubated with graded levels of 12(S)-HpETE for 10 min, before addition of substrate DEVD-pNA. After further 30 min of incubation, the cleavage of DEVD-pNA was measured at 405 nm and expressed as absolute unit. The results represent two independent experiments.*

Critical for apoptotic processes, caspases are cysteine-dependent and sensitive to oxidation, hence, high levels of lipid peroxide from 12-LOX may lead to their inactivation. To study this possibility, we examined whether 12(S)-HpETE can inhibit the activity of Caspase-3, an effector caspase, which can cleave a broad spectrum of cellular targets. As shown in **Figure 10**, 12(S)-HpETE inhibited caspase-3 activity in a dose-dependent manner.

Task 2. ***Determine whether or not stimulation of VEGF is required by 12-LOX to enhance radioresistance in vitro and in vivo.***

We will use a VEGF neutralizing antibody to study whether VEGF is required for 12-LOX mediated radioresistance in PC-3 cells. Matrigel implantation model will be used to assess 12-LOX mediated radioresistance in vivo and to study the role of VEGF in this process. This task has been initiated, with the following preliminary findings:

To study whether 12-LOX can regulate VEGF expression, we measured VEGF levels in culture supernatants from 12-LOX transfected PC-3 cells (nL-8 and nL-12) and their vector controls. As shown in **Figure 11**, increased expression of 12-LOX enhanced VEGF expression. Northern blot analysis revealed an increase in the levels of VEGF mRNA in 12-LOX transfected PC-3 cells (nL-2 and nL-8) (**Figure 12)**.

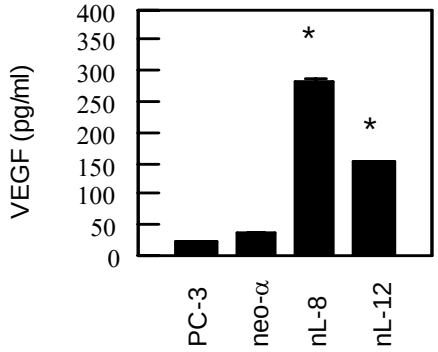


Figure 11*. Increased VEGF Expression in 12-LOX Transfected PC-3 Cells. *, P < 0.01.*

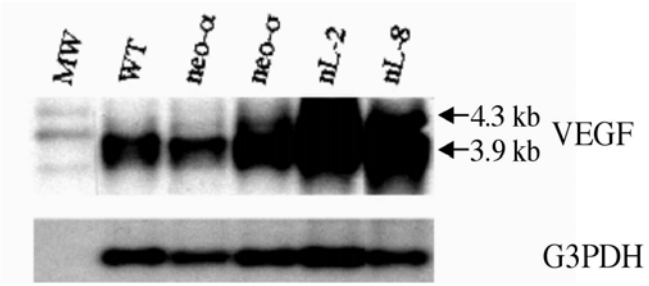


Figure 12. *Northern Blot Analysis of VEGF mRNA Levels. Poly(A)+RNA were isolated and the 12-LOX mRNA levels were analyzed with labeled VEGF cDNA. The membrane was then stripped and probed for G3PDH as a loading control.*

As part of our effort to study how prostate cancer cells survive clonogenicaly from radiation treatment, we subjected PCa cells to radiation treatment (800 cGy) and the clonogenically survived cells were isolated and propagated. As shown in **Figure 13**, a subline of DU145 cells (DU10a), presented a much higher resistance to subsequent radiation treatment. Similar results were also obtained in PC-3 cells. The data suggest that prostate cancer cells, after surviving potential lethal dose of radiotherapy, become more resistant to subsequent radiation treatment.

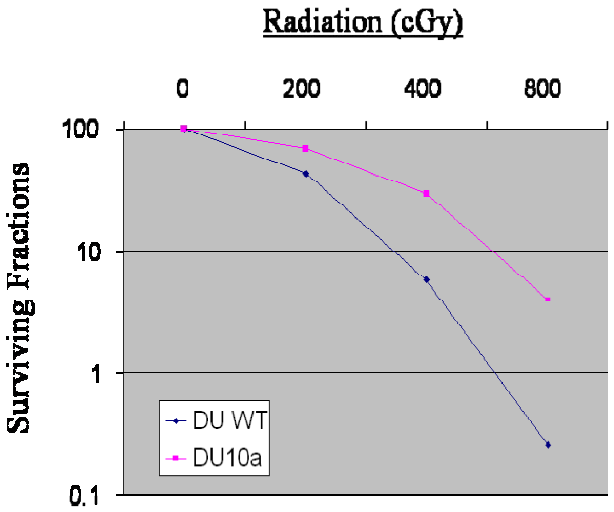


Figure 13*. Increased radioresistance of DU145 cells after surviving radiotherapy. DU10a are DU145 cells that have survived a potential lethal dose of radiation (800 cGy). DUWT are parental DU145 cells that have survived from sham radiation.*

Next we examined whether there is a change in NF-κB activity, which is known for its role in angiogenesis and tumor growth, in tumor cells surviving radiation treatment. As shown in **Figure 14**, there was a four fold of increase in NF-κB promoter activity in DU10a, as compared to the control, DU145 cells. The data suggest that there is a *sustained* elevation in NF-κB activity in PCa cells that clonogenically survived from radiation treatment.

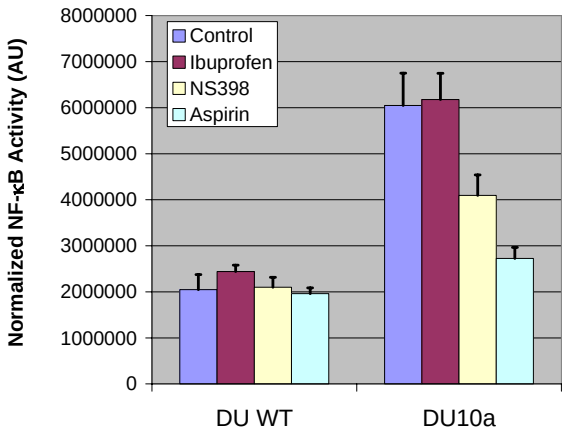


Figure 14*. Increased NF-κB activity in clonogenically survived DU145 cells (DU10a) and its downregulation by aspirin and NS398. Cells were transfected with a reporter gene construct under NF-kB promoter and treated with ethanol (control), ibuprofen (1 mM), NS398 (20 μM), or aspirin (0.5 mM) for 18 h before luciferase assay. Note the downregulation of NF-κB activity in DU10a cells by NS398 and aspirin.*

Interestingly, the sustained activation of NF-κB in radioresistant DU10a cells was markedly reduced by aspirin treatment (**Figure 14**). The ability of this widely used OTC drug to downregulate the sustained activation of NF-κB in clonogenically survived PCa cells raises an exciting possibility, of using inhibitors of NF-κB, such as sodium salicylates (aspirin), to overcome PCa radioresistance.

Radiation has been demonstrated to activate NF-κB as part of the immediate early response. We also found that IR can increase NF-κB promoter activities in prostate carcinoma PC-3 and DU145 cells in a dose dependent manner (data not shown) and this activation was associated by a reduction in the level of IκBα (**Figure 15**).

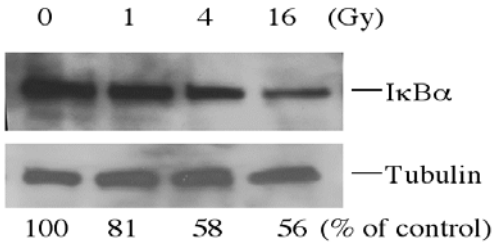


***Figure 15**. Reduction of IκBα levels in prostate carcinoma DU145 cells after IR. Shown here is the level of IκBα level 16 h after IR as revealed by western blot. The level of tubulin was included as a loading control.*

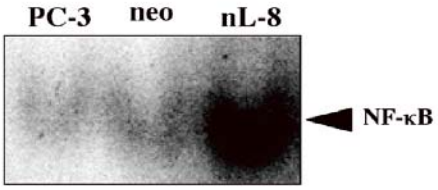


***Figure 16.** Effect of 12-lipoxygenase overexpression on NF-κB activity. EMSA was performed on nuclear extracts of untransfected PC-3, neo, and 12-LOX transfected cells (nL8).Note the increase in NF-κB DNA binding activity in nL8.*

Radiation also rapidly increases the enzymatic activity of 12-LOX. We examined whether or not 12-lipoxygenase regulates NF-κB activity in prostate cancer cells using electomobility shift assays (EMSAs), western blotting for IκBα, and transcriptional activity with luciferase reporter assay. Nuclear protein extracts of 12-LOX transfected cells (nL-8) showed significant constitutive activation of NF-κB compared to the vector control cells (neo-α) or the untransfected PC-3 cells (**Figure 16**).

This activation was further confirmed by the increased transcriptional activity of the luciferase reporter construct, driven by NF-κB, in nL-12 cells (**Figure 17**). This increase in transcriptional activity observed in 12-LOX transfected cells was nearly abolished upon co-transfection of a mutant of IκBα that is resistant to proteolytic degradation purchased from Upstate Biotechonogy (Lake Placid, NJ) (**Figure 17**). Activation of NF-κB involves phosphorylation and eventual degradation of IκB protein before NF-κB could bind to DNA. Western blot analysis of whole cell protein extracts from neo-α, nL-8, and nL-12 cells showed a dramatic decrease in IκBα in nL-8 and nL-12 cells (**Figure 18**). These results strongly suggest that overexpression of 12-LOX induces NF-κB activity by a mechanism involving proteolytic degradation of IκBα.

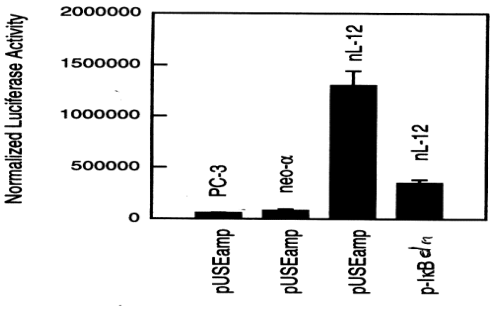


Figure 17. *Effect of 12-lipoxygenase overexpression on NF-κB activity. The cells (PC-3, neo, and a clone of 12-LOX transfected PC-3, nL-12) were transfected with NF-κB-luciferase and LacZ reporters (pUSEamp) and the reporter activities measured. To elucidate the role of of IκBα in 12-LOX activation of NF-κB, cells were also transfected with a dominant negative mutant IκBα construct (p-IκBdn), or its control vector pUSEamp. The normalized data shown is an average of three experiments with standard deviation. LacZ expression was used for normalization.*

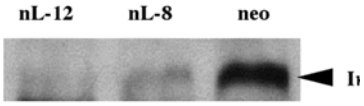


Figure 18. *Reduction of IκBα levels by overexpression of 12-LOX in PC-3 cells. The levels of IκBα were evaluated by western blot analysis in cell lysates from 12-LOX overexpressing PC-3 cells (nL-8 and nL-12) and their vector control (neo).*

Next we used BHPP, a select inhibitor of 12-LOX (Nie et al., 2000), to study the role of the enzymatic activity of 12-LOX in NF- κB activation. DNA binding activity of NF-κB was greatly decreased upon exposure to 20 μM BHPP for 60 min (**Figure 19**). The results show the participation of the 12-LOX enzymatic activity in NF- κB activation.

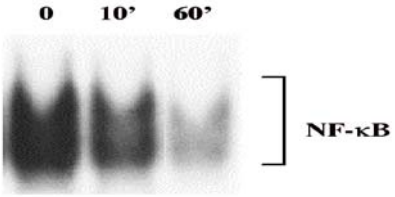
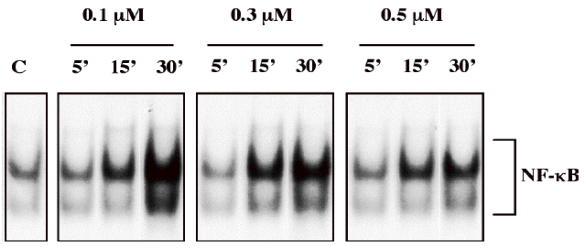


Figure 19. *Inhibition of NF-κB activation in 12-Lipoxygenase transfected PC-3 cells (nL8) by BHPP, a select 12-LOX inhibitor*

To further study the involvement of 12(S)-HETE in the activation of NF-κB, we evaluated the DNA binding activity of NF-κB when PC-3 cells were treated with 12(S)-HETE. As shown in **figure 20**, 12(S)-HETE can modulate NF-κB DNA binding activity in a dose- and time-dependent manner. Further, the increased NF-κB DNA binding activity was accompanied by the nuclear translocation of NF-κB from cytosol to the nucleus, as revealed by immunocytochemical analysis of the p65 subunit of NF-κB (**Figure 21**).



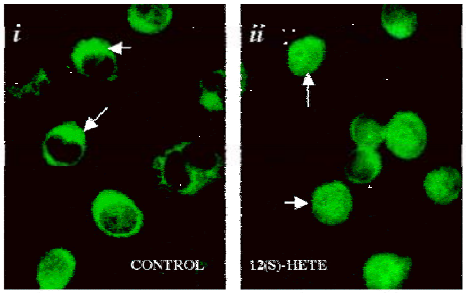


Figure 20. *Effect of 12(S)-HETE on the activation of NF-κB*

Figure 21: *Immunofluorescent localization of NF-κB in PC-3*

in PC-3 cells. Time and dose dependent activation of NF-κB DNA binding activity by 12(S)-HETE. EMSA was performed on the nuclear extracts of the treated PC-3 cells. The cells were incubated with serum-free RPMI medium containing the amounts of 12(S)-HETE shown for the indicated time and subjected to EMSA.

*cells with and without 12(S)-HETE treatment. Cells were treated with 100 nM 12(S)-HETE or buffer for 10 min and immunostained. Staining was predominantly present in the cytoplasm of untreated control cells (*Left panel, *arrows) and nuclear staining increased considerably in 12(S)-HETE treated cells (*Right panel, *arrows).*

The pro-angiogenic activity of 12-LOX also can play a role in the recurrence of tumor cells at sites of irradiation. The proangiogenic and radioresistant activity of 12-LOX bears a similarity to VEGF, a putative angiogenic factor that is also involved in clonogenic survival of tumor cells after radiation. We thus examined the relationship between 12-LOX and VEGF in PCa cells after radiation. ELISA analysis of the culture supernatants in PC-3 cells 24 hours after irradiation indicated an upregulation of VEGF levels at clinically relevant dosages (Figure, 200 and 400 cGy). Treatment of cells with baicalein (20 μM) abolished the stimulation of VEGF expression by radiation (**Figure 22**), suggesting an important role for 12-LOX in radiation-stimulated VEGF expression.

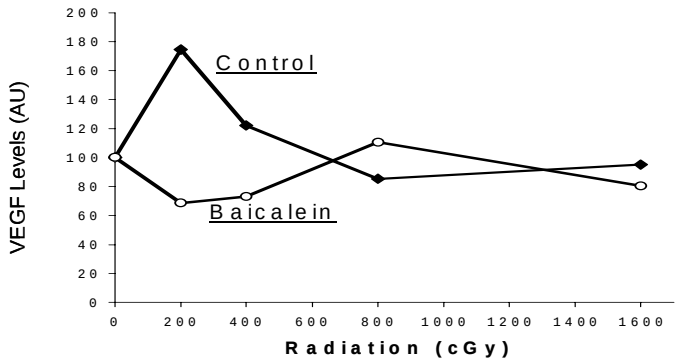


***Figure 22**. Stimulation of VEGF production by low dose radiation in PC-3 cells and its dependence on 12-LOX activity.*

Task 3. ***Evaluate whether or not 12-LOX inhibitor BHPP can sensitize prostate tumors to radiation in vivo.***

To study whether inhibition of 12-LOX can sensitize prostate tumor to radiation in vivo, we injected 2 X 10^6 12-LOX transfected PC-3 cells (P-LOX), mixed with 0.5 ml 10X diluted Matrigel, s.c. in the middle of the back at 1.5 cm from the tail. Diluted Matrigel was used to promote tumor formation and growth in a more consistent and uniform way. After 4 weeks, palpable tumors reached to a size of 0.2 ~ 0.3 cm in diameter. BHPP (50 mg / kg) or control solvent was injected i.p. into mice 2 hours before radiation treatment of xenografted tumors. For irradiation of xenografted prostate tumors, tumor-bearing mice were immobilized in lead jigs containing cut-out windows of 2cm/2cm for exposure of the s.c. tumors on the lower back. Four jigs at a time were positioned on an aluminum frame mounted on the X-ray machine. Radiation was be delivered to the tumors using a Siemens Stabilipan X ray set operated at 250 kV, 15 mA with 1 mm copper filter at a distance of 28 cm from the start for a single dose of 600 cGy (Sheldon and Hill, 1977; Kjellen et al., 1991). After radiation treatment, the mice were returned to cage and the growth of tumors were monitored closely and tumor sizes measured three times a week. As shown in **Figure 23**, radiation caused a reduction in tumor growth (Rad vs Non-rad). Pretreatment of tumor bearing mice further reduced tumor growth after radiotherapy (Rad vs. BHPP-rad), suggesting that inhibition of 12-LOX may be a promising approach to enhance the efficacy of radiotherapy for prostate tumors in vivo.

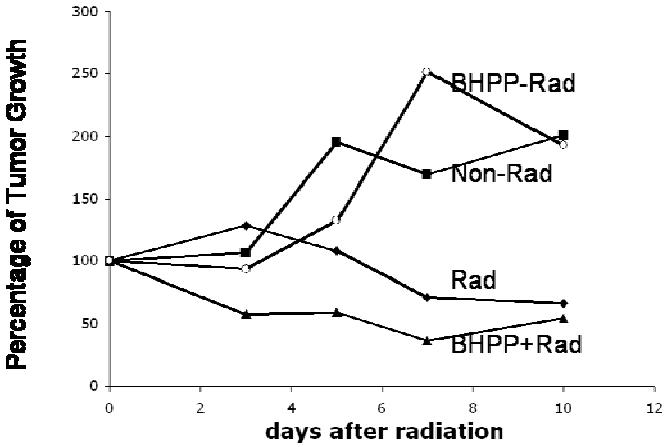


***Figure 23**. Effects of 12-LOX inhibitor BHPP on radiation response of prostate tumor in vivo.*

We also tested the radiosensitizing activities of BHPP at different concentrations and regimens. 10^6 PC3 cells were inoculated into SCID mice, were left to growth to a size of 100 mm^3 (day 16) and were treated with 12-LOX inhibitor, BHPP i.p. (100 μg/kg) for 4 consecutive days or irradiated on day 16 or the two modalities were combined. The changes in tumor volume were monitored untill day 28. Data indicated that 4 days BHPP administration alone did not affect s.c. growth of PC3 cells, but single administration of 5 Gy irradiation caused a significant delay in tumor growth between days 21 and 25. However, the most effective growth inhibition was detected in the combination treatment group: where the inhibitory effect manifested from day 25.

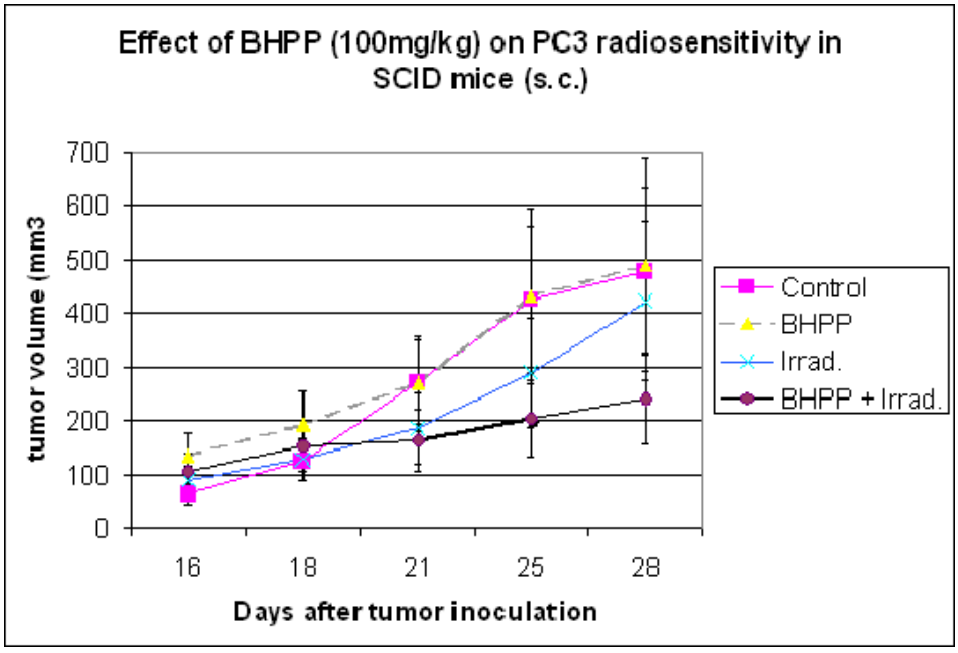


***Figure 23**. Effects of 12-LOX inhibitor BHPP on radiation response of prostate tumor in vivo.*

In PC-3 cells the combined effect of radiation and 12-LOX inhibitors Baicalein and BHPP proved to be supraadditive strongly or moderately depending on the combination regimen used as shown by the

combination indexes calculated employing the median effect principle. In case of LNCaP cells the radiation enhancing effect of 12-LOX inhibitors was apparent only from higher – over 100 cGy – radiation doses. In contrast to the hormone sensitive PC-3 and LNCaP cells, the combined radiation and 12-LOX inhibitor treatment of the hormone refractory cell line DU 145 showed only an approximately simple additive effect.

Table 8. Combination indexes calculated employing mutually exclusive assumption and median effect principle by the Calcusyn software

Radiation dose (cGy)	PC-3		LNCaP		DU 145
	25 μM BHPP	7.5 μM Baicalein	25 μM BHPP	7.5 μM Baicalein	25 μM BHPP
25	0.87	0.35	1.23	1.10	1.12
50	0.66	0.57	0.89	1.01	1.05
100	0.64	0.13	0.76	0.87	1.36
200	0.61	0.10	0.56	0.69	1.54
500	0.50	0.11	0.62	0.58	1.23

In PC-3 and LNCaP cells a synergistic effect can be seen, while in DU 145 cells the effect is additive-subadditive. Combination indexes were calculated based on the Chou-Talalay equation. CI=1 represents simple additive, while CI>subadditive, CI<supraadditive effect.

SUMMARY/CONCLUSIONS:

Our studies found that 12-LOX promotes the resistance of prostate cancer cells toward radiation treatment. We also found when combined, 12-LOX inhibitors and radiation had synergistic effects in killing PCa cells and this was accompanied by an increase in the level of the active form of caspase-3. Our studies suggest that 12-LOX inhibitors are promising radiosensitizer and further work need to be done to determine the mechanism of radiosensitization and the efficacy of 12-LOX inhibitors in sensitizing prostate tumors to radiation treatment.

REPORTABLE OUTCOMES

- Four review articles published.
  Nie D, Honn KV.Eicosanoid regulation of angiogenesis in tumors. Semin Thromb Hemost. 2004 30:119-25.

  Nie D. Cyclooxygenases and lipoxygenases in prostate and breast cancer. Frontier in Bioscience 12: 1574-1585, 2007.

  Wang MT, Honn KV, and **Nie D**. Cyclooxygenases, prostanoids, and tumor progressions. Cancer Metastasis Rev. 26: 525-534, 2007.

  Pidgeon GP, Lysaght J, Krishnamoorthy S, Reynolds JV, O'byrne K, **Nie D**, Honn KV. Lipoxygenase metabolism: roles in tumor progression and survival. Cancer Metastasis Rev. 26: 503-524, 2007.

  Tang Y, Olefumi L, Wang M, **Nie D**. Role of Rho GTPases in Breast Cancer. Frontiers in Biosciences 13: 759-776, 2008.

- Three research articles published
  Nie D, Krishnamoorth S, Jin R, Tang K, Chen U, Qiao Y, Zacharek A, Guo Y, Milanini J, Pages G, Honn KV. Mechanisms regulating tumor angiogenesis by 12-lipoxygenase in prostate cancer cells. Journal of Biological Chemistry 281: 18601 – 18609, 2006.

  Chen Y, Tang Y, Zeng S, **Nie D**. Human Pregnane X Receptor and Resistance to Chemotherapy in Prostate Cancer. Cancer Research 67: 10361-10367, 2007.

  Nie D, Guo Y, Yang D, Tang Y, Chen Y, Zacharek A, Yang Q, Che M, Honn KV. Thromboxane A2 Receptors in Prostate Carcinoma: Expression and its Role in Regulating Cell Motility via Small GTPase Rho. Cancer Research 68: 115-121, 2008.

- One research article to be submitted
  Nie D., J Lovey, MT Wang, K Tang, J Timar, KV Honn. Regulation of prostate cancer radioresponses by 12-lipoxygenase. To be submitted.

- Abstract published.
  Krishnamoorthy, S., K. R. Maddipati, D. Nie, and K. V. Honn. 12-Lipoxygenase in hypoxia and hypoxia-induced angiogenesis. Proc. Amer. Assoc. Cancer Res. 45: #3591, 2004.
- Abstract published.

Nie, D., Y. Qiao, A. Zacharek, and K. V. Honn. Blockade of NF-κB sensitizes prostate cancer cells to ionizing radiation. Proc. Amer. Assoc. Cancer Res. 45: #1290, 2004.

- Abstract published.
  Wang MT and **Nie D**. Cancer stem cells of prostate in resistance to radiotherapy. Proceedings of US Department of Defense IMPaCT Meeting (Atlanta, GA. September, 2007).

- Patent applied. A provisional patent application, entitled "12-Lipoxygenase inhibitors as radiosenstizer for prostate cancer" has been filed.

- Development of animal models: No.